

Product Information

Microsomes, pooled from female human liver

Product Number **M 0692**
Storage Temperature $-70\text{ }^{\circ}\text{C}$

Product Description

Liver microsomes are subcellular particles derived from the endoplasmic reticulum of hepatic cells. These microsomes are a rich source of drug metabolizing enzymes, including cytochrome P450. Microsome pools from various sources are useful in the study of xenobiotic metabolism and drug interactions.

This product contains a mixture of liver microsomes pooled from different individual human donors. The pathogenicity testing of all liver specimens has been performed using a PCR protocol. The donors are human females of mixed age. The donors are in various states of health, however each liver tested negative for HIV1&2, HTLV1&2, and hepatitis B and C.

The protein content determined by the Lowry method is a minimum of 20 mg/ml in 250 mM sucrose and is specifically reported on the certificate of analysis (C of A). Total cytochrome P450, oxidoreductase, cytochrome b₅, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP4A, and FMO activities are also reported on the lot specific C of A.

Precautions and Disclaimer

This product is for laboratory research use only. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

1. Quickly thaw at $37\text{ }^{\circ}\text{C}$ using a water bath. Keep on ice until ready to use.
2. If not using the entire contents, aliquot to minimize freeze-thaw cycles.
3. Store aliquots at $-70\text{ }^{\circ}\text{C}$.

Storage/Stability

The product is shipped on dry ice and it is recommended to store the product at $-70\text{ }^{\circ}\text{C}$. The product, as supplied, is stable for at least 2 years if stored properly.

Product Profile

Total cytochrome P450 and cytochrome b₅ activities are assayed by the standard method of Omura and Sato.¹

Enzyme activities on the product are determined as follows:

Oxidoreductase Activity:

Determined as cytochrome c reductase activity. The reaction is initiated by the addition of 0.1 mg/ml protein to 1.0 ml of reaction mixture containing 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase, 3.3 mM MgCl₂, and 0.95 mg/ml cytochrome c in 0.25 M potassium phosphate buffer, pH 7.4, at $37\text{ }^{\circ}\text{C}$. The absorbance change at 550 nm is recorded as a function of time. An extinction coefficient for reduced (ferrous) cytochrome c at 550 nm of $19.6\text{ mM}^{-1}\text{ cm}^{-1}$ is used to calculate the reductase activity. One unit will reduce 1 nanomole of cytochrome c per minute at pH 7.4 at $37\text{ }^{\circ}\text{C}$.

CYP1A2 Isozyme Activity:

Determined as phenacetin O-deethylase activity. Incubations are conducted at 0.8 mg/ml protein with 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase, and 3.3 mM MgCl₂ in 0.1 M potassium phosphate buffer, pH 7.4, for 20 minutes. One unit will produce 1 picomole of acetamidophenol per minute at pH 7.4 at $37\text{ }^{\circ}\text{C}$.

CYP2D6 Isozyme Activity:

Determined as bufuralol 1'-hydroxylase activity. Incubations are conducted at 0.8 mg/ml protein with 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase, and 3.3 mM MgCl₂ in 0.1 M potassium phosphate buffer, pH 7.4, for 20 minutes. One unit will produce 1 picomole of 1'-hydroxybufuralol per minute at pH 7.4 at $37\text{ }^{\circ}\text{C}$.

CYP2E1 Isozyme Activity:

Determined as chlorzoxazone 6-hydroxylase activity. Incubations are conducted at 0.8 mg/ml protein with 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase, and 3.3 mM MgCl₂ in 0.1 M potassium phosphate buffer, pH 7.4, for 20 minutes. One unit will produce 1 picomole of 6'-hydroxychlorzoxazone per minute at pH 7.4 at 37 °C.

CYP2A6 Isozyme Activity:

Determined as coumarin 7-hydroxylase activity. Incubations are conducted at 0.8 mg/ml protein with 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase, and 3.3 mM MgCl₂ in 0.1 M Tris, pH 7.5, for 20 minutes. One unit will produce 1 picomole of 7-hydroxycoumarin per minute at pH 7.5 at 37 °C.

CYP2B6 Isozyme Activity:

Determined as (S)-mephenytoin N-demethylase activity. Incubations are conducted at 0.8 mg/ml protein with 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase, and 3.3 mM MgCl₂ in 0.05 M potassium phosphate buffer, pH 7.4, for 20 minutes. One unit will produce 1 picomole of nervalol per minute at pH 7.4 at 37 °C.

CYP2C19 Isozyme Activity:

Determined as (S)-mephenytoin 4'-hydroxylase activity. Incubations are conducted at 0.8 mg/ml protein with 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase, and 3.3 mM MgCl₂ in 0.05 M potassium phosphate buffer, pH 7.4, for 20 minutes. One unit will produce 1 picomole of 4'-hydroxymephenytoin per minute at pH 7.4 at 37 °C.

CYP2C8 Isozyme Activity:

Determined as paclitaxel 6 α -hydroxylase activity. Incubations are conducted at 0.8 mg/ml protein with 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase, and 3.3 mM MgCl₂ in 0.1 M potassium phosphate buffer, pH 7.4, for 10 minutes. One unit will produce 1 picomole of 6 α -hydroxypaclitaxel per minute at pH 7.4 at 37 °C.

CYP2C9 Isozyme Activity:

Determined as diclofenac 4'-hydroxylase activity. Incubations are conducted at 0.8 mg/ml protein with 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase, and 3.3 mM MgCl₂ in 0.1 M Tris, pH 7.5, for 10 minutes. One unit will produce 1 picomole of 4'-hydroxydiclofenac per minute at pH 7.5 at 37 °C.

CYP4A Isozyme Activity:

Determined as lauric acid 12-hydroxylase activity. Incubations are conducted at 0.8 mg/ml protein with 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase, and 3.3 mM MgCl₂ in 0.1 M Tris, pH 7.5, for 10 minutes. One unit will produce 1 picomole of 12-hydroxylauric acid per minute at pH 7.5 at 37 °C.

CYP3A4 Isozyme Activity:

Determined as testosterone 6 β -hydroxylase activity. Incubations are conducted at 0.5 mg/ml protein with 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase, and 3.3 mM MgCl₂ in 0.1 M potassium phosphate buffer, pH 7.4, for 10 minutes. One unit will produce 1 picomole of 6 β -hydroxytestosterone per minute at pH 7.4 at 37 °C.

FMO Activity:

Determined as methyl p-tolyl sulfide oxidase activity. Incubations are conducted at 0.8 mg/ml protein with 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase, and 3.3 mM MgCl₂, 1.2 mM diethylenetriaminepentacetic acid, 0.5 mg/ml TRITON[®] X-100 in 0.05 M glycine buffer, pH 9.5, for 10 minutes. One unit will produce 1 picomole of methyl p-tolyl sulfoxide per minute at pH 9.5 at 37 °C.

References

1. Omura, T., and Sato, R., J. Biol.Chem., **239**, 2379, (1964).

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